

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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FILING DATE:	§	
	§	
APPLICANT: Chapkin, et al.	§	EXAMINER:
	§	
TITLE: INVASIVE DETECTION	§	
OF COLONIC BIOMARKERS	§	

**Box Patent Application**  
**Assistant Commissioner of Patents and Trademarks**  
**Washington, DC**

**PRELIMINARY AMENDMENTS**

Dear Sir:

Applicants respectfully request that the below amendments be made prior to examination of this application. The application has not yet received a serial number, however, it was filed on May 14, 2001.

**In the specification:**

Please replace the title with the following substitute title. Applicants have included a marked version attached as Appendix A to illustrate the changes contained herein.

INVASIVE DETECTION OF COLONIC BIOMARKERS

After the title, please insert the following paragraph:

"This application is a divisional application of United States Application 09/381,086 was filed September 14, 1999, which was a continuation -in-part of PCT Application No. PCT/US98/06698 filed April 3, 1998, which claims priority to United States Provisional Patent Application No. 60/043,048 filed April 4, 1997.

**In the claims:**

Please cancel, without prejudice and without acquiescence, claims 1-33.

Please enter the following new claims:

34. A method for invasively determining the expression of PKC isozymes in colonocytes of a patient comprising:

directly isolating from said patient polyA+RNA from rectal vault eluate, containing sloughed colonocytes; and

assaying the isolated polyA+RNA and determining the level, in the isolated A+RNA, of mRNA encoding at least one PKC isozyme, wherein the PKC isozyme is PKC  $\zeta$  and PKC  $\beta$ II.

35. The method of claim 34, wherein the level of expression of PKC isozymes PKC  $\zeta$  and PKC  $\beta$ II is determined.

36. The method of claim 35, wherein the ratio of expression PKC  $\beta$ II to PKC  $\zeta$  is determined.

37. The method of claim 36, further comprising the step of comparing the ratio of expression of PKC  $\beta$ II to PKC  $\zeta$  in said patient with similarly determined ratios of PKC  $\beta$ II to PKC  $\zeta$  in other patients with known conditions.

38. The method of claim 37, wherein the level of expression of PKC  $\beta$ II to PKC  $\zeta$  in said patient is compared with similarly determined ratios of PKC  $\beta$ II to PKC  $\zeta$  in at least two other patients, one with colon cancer and one without colon cancer.

39. The method of claim 35, wherein the level of PKC  $\zeta$  is determined using the primer pair having Sequence ID Numbers 7 and 8, and the level of PKC  $\beta$ II is determined using the primer pair having Sequence ID Numbers 11 and 12.

40. A method for invasively detecting colonic biomarkers in a patient using rectal vault eluate messenger RNA comprising:

directly isolating, from said patient, polyA+RNA from rectal vault eluate containing sloughed colonocytes; and

assaying the isolated polyA+RNA and determining the level, in the isolated polyA+RNA, of mRNA encoding at least one colonic biomarker, wherein the colonic biomarker is PKC  $\zeta$  and PKC  $\beta$ II.

41. The method of claim 40, wherein the level of expression of PKC isozymes PKC  $\zeta$  and PKC  $\beta$ II is determined.

42. The method of claim 41, wherein the ratio of expression PKC  $\beta$ II to PKC  $\zeta$  is determined.

43. The method of claim 42, further comprising the step of comparing the ratio of expression of PKC  $\beta$ II to PKC  $\zeta$  in said patient with similarly determined ratios of PKC  $\beta$ II to PKC  $\zeta$  in at least two other patients, one with colon cancer and one without colon cancer.

44. The method of claim 41, wherein the level of PKC  $\zeta$  is determined using the primer pair Sequence ID Numbers 7 and 8, and the level of PKC  $\beta$ II is determined using the primer pair having Sequence ID Numbers 11 and 12.

45. A method for invasively screening for colon cancer in a patient comprising:

detecting the expression of PKC  $\zeta$  and PKC  $\beta$ II in sloughed colonocytes in said patient's rectal vault eluate; and

correlating the expression of PKC  $\zeta$  and PKC  $\beta$ II with the presence or absence of colon cancer in said patient.

46. The method of claim 45, wherein the level of expression of PKC isozymes PKC  $\zeta$  and PKC  $\beta$ II is determined.

47. The method of claim 46, wherein the ratio of expression PKC  $\beta$ II to PKC  $\zeta$  is determined.

48. The method of claim 47, further comprising the step of comparing the ratio of expression of PKC  $\beta$ II to PKC  $\zeta$  in said patient with similarly determined ratios of PKC  $\beta$ II to PKC  $\zeta$  in at least two other patients, one with colon cancer and one without colon cancer.

49. The method of claim 46, wherein the level of PKC  $\zeta$  is determined using the primer pair having Sequence ID Numbers 7 and 8, and the level of PKC  $\beta$ II is determined using the primer pair having Sequence ID Numbers 11 and 12.

REMARKS

Claims 1-33 were filed in the original application. Claims 1-33 have been canceled and claims 34-49 have been added to this divisional application. Support for Claims 34-49 can be found on page14-15 of the Specification. Applicants assert that no new matter was added.

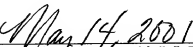
If the Examiner has any questions, please contact the undersigned below.

Respectfully submitted,



Melissa W. Acosta  
Registration No. 45,872

Date:



Fulbright & Jaworski, L.L.P.  
1301 McKinney, Suite 5100  
Houston, Texas 77010-3095  
(713) 651-5407 Telephone  
(713) 651-5246 Facsimile

## APPENDIX A SHOWING MARKED CHANGES

[NONINVASIVE] INVASIVE DETECTION OF COLONIC BIOMARKERS  
[USING FECAL MESSENGER RNA]